REMARKS

Entry of the foregoing and prompt and favorable consideration of the subject application, in light of the following remarks, are respectfully requested.

By the foregoing amendment, the specification has been amended to insert the information concerning the continuing and foreign priority data, to insert the paper copy of the Sequence Listing, to insert the Abstract on a separate sheet of paper and to make the disclosure consistent with regard to amendments made to the specification in the parent application. Furthermore, originally presented (and renumbered) claims 1-51 have been canceled without prejudice or disclaimer and new claims 52-101 have been added. Support for these new claims can be found throughout the originally filed application. Hence, no new matter has been added.

In the event that there are any questions relating to this Preliminary Amendment, or the application in general, it would be appreciated if the Examiner would telephone the undersigned attorney concerning such questions so that prosecution of this application may be expedited.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: _____
Susan M. Dadio
Registration No. 40,373

P.O. Box 1404
Alexandria, Virginia 22313-1404
(703) 836-6620
Date: December 7, 2001

Attachment to Preliminary Amendment dated December 7, 2001

Marked-up Copy

Page 6, Paragraph Beginning at Line 11

[Figure 12 shows] Figures 12A and 12B show in schematic form the construction of plasmid pSM215.

Panel A shows a map of mouse GAD65 cDNA.

Panel B shows a map of expression vector pSM215.

Page 17, Paragraphs Beginning at Line 16 through Page 17, line 37

The signal sequence was isolated from barley α-amylase cDNA clone as a PCR product using two synthetic primers. The forward primer (5' -CGGATCCGGCGCGC GCCATGGGGAAG - 3') (SEQ ID NO.:1) had a BamHI site added to 5' end to facilitate cloning, and the reverse primer (5' -GGAATTCCCGGGCGCCGGACGCCAAAC CCGGCGAG - 3') (SEQ ID NO.:2) contained two engineered restriction sites, EcoR1 and NarI. EcoR1 was used for convenience in subcloning whereas Nar1 provided a site for fusion. The PCR product was isolated, digested with BamHI and EcoR1, and cloned into pBluscriptII (Stratagene, an E. coli plasmid vector which does not have any NarI site), to form intermediate plasmid pBluscriptII-10.

The DNA fragment encoding the mature peptide sequence (native protein minus signal peptide) of murine II α chain was created by PCR using the following two synthetic

primers: 5'- GGGCGCCGAAGACGACATTGAGGCCGAC - 3' (SEQ ID NO.:3) (forward reaction), which contained a compatible NarI site at its 5', and 5' - CGAATT CTCATAAAGGCCCTGGGTGTCT - 3' (SEQ ID NO.:4) (reverse reaction) which had an EcoR1 site attached to the 5' end. The PCR product was rescued as an EcoR1 + NarI fragment.

Page 18, Paragraph Beginning at Line 10

pSM156 was constructed by replacing the native signal sequence of I-A β gene with the signal sequence of barley α-amylase (Figure 2). The strategy employed was essentially the same as for the construction of pSM155. Two primers were used for the isolation of mature β gene coding sequence: 5' - GGGCGCCGAAGACGACATTGAGGCCGAC - 3' (SEQ ID NO.:5) (forward primer) and 5' CGAATTCTCATAAAGGCCCTGGGTGTCT - 3' (SEQ ID NO.:6) (reverse primer).

Page 18, Paragraph Beginning at Line 20

CONSTRUCTION OF pSM151-del: pSM151-del contains the truncated form of I-A α gene in which its DNA sequence determining the C-terminal cytoplasmic domain, was deleted, as in Figure 1. This was obtained by polymerase chain reaction-mediated amplification after a 1.1kb EcoR1 fragment was cloned into pUC19. The M13/PUC universal primer (5' - GTAAAACGACGGCCAGT-3') (SEQ ID NO.:7) is used as a forward primer. The reverse primer (5' -CGAATTCTCACAGGCCTTGAA

TGATGAAGAT-3') (SEQ ID NO.:8) corresponding to I-A α encoding sequence between nucleotides 715 and 732, introduces a termination codon TGA starting at nucleotide position 733, followed by an EcoR1 cloning site. The truncated gene was amplified by 25 cycles of heating (94°C, 1min), annealing (55°C, 1.5min), and extension (72°C, 2min). The reaction product was purified, digested with EcoR1, blunt-ended with Klenow fragment, and first inserted into pSM150, and then the whole expression cassette was reisolated as a EcoR1 and HindIII fragment and subcloned into pBIN19 to give pSM151-del (Figure 2).

Page 19, Paragraph Beginning at Line 3

CONSTRUCTION OF pSM152-del: pSMA152-del contains the truncated I-A β gene which has its DNA sequence determining the C-terminal cytoplasmic domain removed (Figure 2). This was accomplished essentially by the same procedure as used to construct PSM151-del. The M13/pUC universal primer was used as a forward primer. The reverse primer (5' - CGAATTCTCAGATGAAAAGGCCAAGCCCGAG-3') (SEQ ID NO.:9) which is complementary to the nucleotide sequence of I-A β gene at positions 715 and 735, introduced a TGA stop codon after nucleotide 715, followed by the same EcoR1 cloning site.

Page 22, Paragraph Beginning at Line 17

A plasmid expression vector, pSM215, was constructed as shown in Figure 12. A NcoI restriction site as indicated in Panel A was created by site-directed mutagenesis and used as part of a translational start site. Site-directed mutagenesis was done using the reaction kit purchased from Pharmacia following manufacturer's instructions. The primer used was 5' - GACCACCGAGCCATGGCATCTTC-3' (SEQ ID NO.:10) which includes a new NcoI restriction site. The modified murine DNA was cloned into plasmid pSM150. The translation start (ATG) and stop (TGA) sites are indicated. This GAD cDNA was inserted between the cauliflower mosaic virus 35S promoter, Ehn 35S, and the transcription termination sequence of nopaline synthase (NOS-ter).